PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of

HANAI, *et al.*

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For: ANTI-FIBROBLAST GROWTH FACTOR-8 MONOCLONAL ANTIBODY

DECLARATION UNDER 37 C.F.R. §1.132

Commissioner for Patents

P.O. Box 1450

Alexandria, VA 22313-1450

Sir:

I, Dr. Kenya SHITARA, a citizen of Japan, do hereby declare as follow:

I graduated from the University of Tokyo, Faculty of Pharmaceutical Science in 1982, entered the graduate school of the University of Tokyo immediately after graduated, and got MSc degree in 1984. My major subject in the University of Tokyo was immunology. Since 1984, I have worked at Tokyo Research Laboratories, Kyowa Hakko Kogyo Co., Ltd. At Tokyo Research Laboratories, I have been studying on establishment and evaluation of anti-tumor monoclonal antibodies. I got Ph.D. degree from the University of Tokyo, Faculty of Pharmaceutical Science in 1990. During 1993 and 1994, I stayed Neurobiology Program, La Jolla Cancer Research Foundation (Present name is The Burnham Institute) U.S.A. and studied on function of the novel proteoglycan in the brain in the lab. From 1997 to 2005, I was Head senior researcher of Division of Immunology, Tokyo Research Laboratories, Kyowa Hakko Kogyo Co., Ltd. Since 2005, I have been a director of Department of Antibody Research, Pharmaceutical Research Center, Kyowa Hakko Kogyo Co., Ltd. I belong to the Japanese Association for Cancer Research, and the Japanese, Association for Metastasis Research. The number of my oral and poster presentations in the academic meetings of these and other international societies, such as the International Conference on AACR-NCI-EORTC, International

Conference on AACR and International Symposium on Cancer Chemotherapy are more than twenty in total. My publications in academic journals are more than fifty; these journals include Journal of Biological Chemistry, Cancer Research, Journal of Immunology, Blood, Oncogene, Proceedings of National Academy of Science USA, etc.

I am familiar with the prosecution history of the above-identified patent application.

The following experimentation was conducted in order to demonstrate the unexpected effects of the present invention.

Experiment

Effects of anti-Flt-1 IgG1 antibodies generated by CHO/DG44 cells on antibody-dependent cellular cytotoxicity.

We evaluated ADCC activity of human CDR-grafted anti-Flt-1 IgG1 antibodies generated by CHO/DG44 cells. The CHO-produced anti-Flt-1 IgG1 antibody failed to show a significant ADCC activity.

1. Materials and Methods

An expression vector of human CDR-grafted anti-Flt-1 IgG1 antibody was generated according to the specification through page 33 line 19 to page 37 line 26, comprising the CDR of variable (V) region in both heavy (H) chain and light (L) chain of the monoclonal antibody KM1750 (FERM BP-5700) established in the Example 1 of the specification.

The anti-Flt-1 antibody was prepared according to the specification

through page 38 line 23 to page 41 line 22. Briefly, the present experimental methods are as follows. Ten micrograms of the anti-Flt1 IgG1 expression vector were transfected into 1.6 × 10^6 cells of dihydrofolate reductase-deficient CHO cell line CHO/DG44 [Proc. Natl. Acad. Sci. USA 77:4216–4220 (1980)] via electroporation, and the transfectants were selected on the basis of antibody production during stepwise gene amplification in IMDM medium (Invitrogen, Carlsbad, CA) containing 10% (v/v) dialyzed fetal bovine serum and methotrexate (MTX; Sigma-Aldrich, St. Louis, MO) from 0, 50, 200 up to 500 nM. MTX-resistant cells were cultured in serum-free medium EX-CELL™301 (JRH Biosciences, Lenexa, KS) for 7 days and antibodies were purified from the culture media using MabSelect™ (Amersham Biosciences, Piscataway, NJ) and stored in 10 mM citrate buffer pH 6.0 with 0.15 M NaCl. Concentration of the purified antibodies was measured by enzyme-linked immunosorbent assay specific for human IgG1 as previously described [Mol. Immunol. 37:1035–1046 (2000)].

An antibody-dependent cellular cytotoxicity (ADCC) assay for each anti-Flt-1 IgG1 was performed according to the specification of page 51 lines 11-15. Briefly, the present experimental methods are as follows. ADCC assay was performed by the lactate dehydrogenase (LDH) release assay using human peripheral blood mononuclear cells (PBMC) prepared from healthy donors by Lymphoprep (Axis Shield, Dundee, UK) as effector cells. Aliquots of target cells, human T cell leukemia Jurkat cells (RIKEN Cell Bank; RCB0806) were distributed into 96-well U-bottomed plates (1 × 10^4 cells) and incubated with concentrations of 0.05, 0.5, 5, or 50 μg/ml anti-Flt1 IgG1 in the presence of human effector cells (1 × 10^6 cells) at an E:T ratio of 100:1. After incubation at 37°C for 4 hours, the supernatant LDH activity was measured using a nonradioactive cytotoxicity assay kit (Wako, Osaka, Japan). Percent specific cytolysis was calculated according to

the formula: %specific lysis = 100 × (E–S_E–S_T)/(M–S_E) where E is the experimental release (supernatant activity from target cells incubated with antibody and effector cells), S_E is the spontaneous release in the presence of effector cells (supernatant activity from target cells incubated with effector cells), S_T is the spontaneous release of target cells (supernatant activity from target cells incubated with medium alone), and M is the maximum release of target cells (activity released from target cells lysed with 9% Triton X-100).

2. Results

No ADCC activities of CHO/DG44-produced anti-Flt1 IgG1 were observed even at the dose of 100 μg/mL.

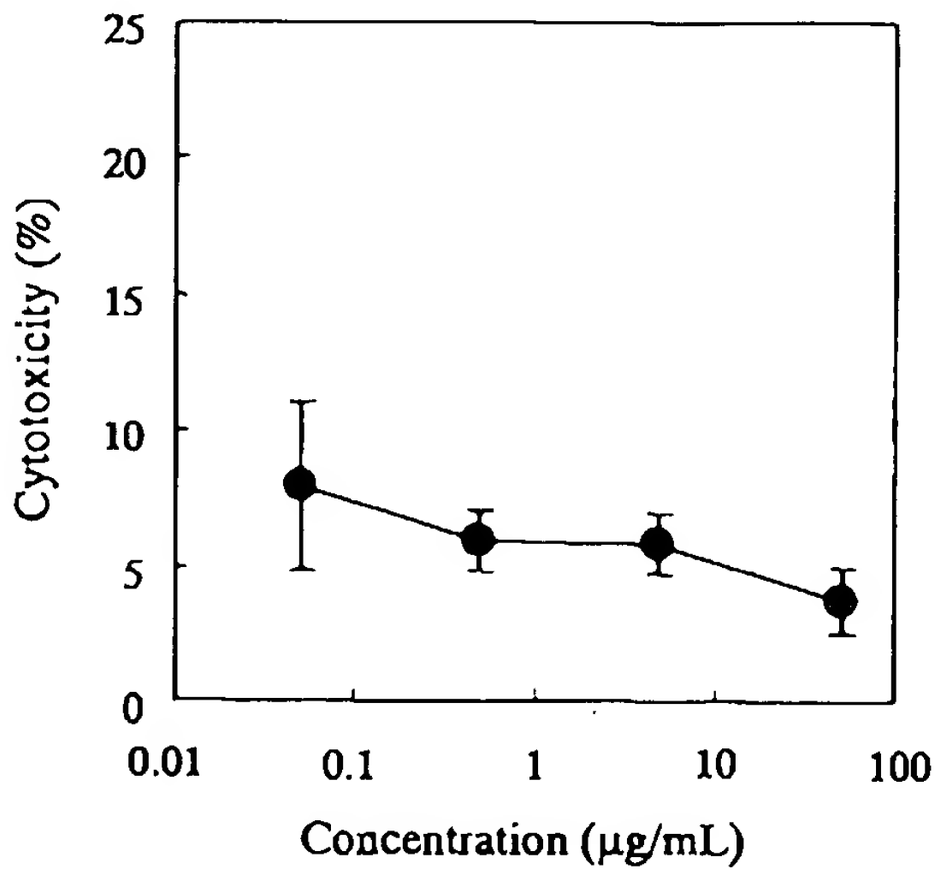

Fig. 1 ADCC activity of anti-Flt-1 antibody

U.S. APPLICATION NO. 10/763,276
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PATENT APPLICATION

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date : Nov. 21, 2006 Name : Kenya Shitara

Dr. Kenya SHITARA